TROUBLESHOOTING GUIDE - IMMUNOPRECIPITATION

Problem	Possible Cause	Possible Solutions
Non-Specific Background	Insufficient washing	• Use more stringent washes. • Alternate wash buffers from high to low salt. • Use a different detergent • Have one wash be with distilled water • Increase the number and time of washes
	Still frozen lysates	• Do not freeze before use
	High antibody concentrations	• Decrease concentration
	Non-specific binding to agarose beads or antibodies	• Pre-clear lysates
	Non-specific binding to Proteins	• Preload precipitated antibody, then block remaining sites with BSA, gelatin, acetone powders or 5% nonfat dry milk
	Aggregated proteins in lysate	• Prior to adding the antibody, thoroughly centrifuge at 100,000 x g for 30 minutes
	Bridging Antibody	• Test the bridging antibody alone by immunoprecipitation
Specific Background	Polyclonal antiserum- protein complexes formed	• Use a monoclonal or affinity purified polyclonal
	Antigen consists of more than one polypeptide chain	• The antigen may already consist of more than one polypeptide chain
	Monoclonal or affinity purified polyclonal antibody recognizing homologous epitope	• Use a monoclonal with a different epitope.
	Immunoblots are actually Ig light or heavy chains	• IgG light chains are recognized at ~28kDa, IgG heavy chains are recognized at ~55kDa
Specific Antigen Not Detected	Non-suitable antibody	• Try a different antibody. Sometimes polyclonals work better
	Antibody concentration too low	• Increase the concentration
	Weak binding to the proteins Too many proteins in mixture	• Use a bridging antibody to capture immunocomplex • Centrifuge lysate at 100,000 x g for another 30 minutes to remove any extra fragments
	Protein only available in low levels in the sample type	• Increase antibody concentration • Increase cell lysate concentration • Metabolically label cellular proteins

Problem	Possible Cause	Possible Solutions
Specific Antigen Not Detected (Cont.)	Other Interfering substances	• Be cognizant of pH, excessive detergent concentrations, and reducing agents such as DTT, and _-mercaptoethanol
	Incorrect bead type used	• Make sure the right ones were used and re-try
	Immune complex was stripped from agarose beads by wash buffer	• Use a milder wash buffer • Change detergents to something with less salt and/or a lower detergent concentration • Reduce number of washes
	Incubation times too short	• Change the incubation time frame so that it can sit overnight at 4ºC
	Antigen destroyed or lost	• Only use fresh lysates that you have prepared yourself